REMARKS

This paper is being filed in response to the Office Action mailed May 5, 2003. Claims 1 to 57 are pending. Elected Group II, claims 1 to 27 and 34 to 57, have been rejoined with Group XI, claims 28 to 33, for examination in the application. Applicants thank the Examiner for rejoining the Group II claims with the Group XI claims. By the present Response, new claims 58 to 83, which depend from claim 4, have been added. Accordingly, upon entry of the Response, claims 1 to 83 and are under consideration.

Regarding the Amendments to the Specification and Sequence Listing

The specification has been amended to insert sequence identifiers (SEQ ID NOs) at pages 4 to 5 and 36 to 46 in accordance with the requirements for sequences under 37 C.F.R. §§1.821-1.825. A marked up copy of the relevant specifciation pages indicating the sequence identifier amendments is submitted herewith. Thus, as the amendments to the specification were made to address informalities, no new matter has been added and entry thereof is respectfully requested.

Regarding the Claim Amendments

The claim amendments are supported throughout the specification or were made to address informalities. In particular, the amendment to claims 1 and 22 conforms the language to the elected invention and, therefore, was made to address an informality. The amendment to claim 3 to recite antibody "subsequence" more clearly indicates antecedent basis in respect to the "subsequence" of claim 2 and, therefore, was made to address an informality. The amendments to claims 2, 4, 22 and 28 are supported, for example, at page 5, lines 13-19, which discloses that "following grafting, one or more amino acids of the antibody are mutated to human sequences," which can be "in a framework region;" and at page 6, lines 8-18, which discloses human antibody "consensus sequence" based upon a survey of "human variable region domain sequences" including "framework regions." The amendment to claim 5 is supported, for example, as set forth above for the amendment to claim 4 and at page 6, lines 2-5, which discloses that humanized antibodies can have "greater or less affinity for the antigen than the donor non-human antibody," and that "affinities range from greater or less affinity for the antigen than either the donor or recombinant antibody;" at page 30, lines 1-5, which discloses

"antibodies that protect against human rhinovirus (HRV) infection," which in one embodiment can have a protective efficacy "greater than the non-humanized antibody;" and at page 34, lines 9-13, which discloses non-human antibody "mouse monoclonal antibody 1A6." The amendment to claim 12 was made to correct a spelling error and to define the invention with greater particularity. The amendment to claims 16 and 17 to also depend from claim 1 is supported, for example, at page 12, lines 6-27, which discloses multispecific and multifunctional antibodies, and at page 8, lines 16-20, which discloses antibody multimers. The amendments to claims 40, 48 and 53 to depend from independent claim 22 instead of dependent claim 21 was made to correct a typographical error and, therefore, addresses an informality. The amendments to claims 47, 50 and 55 were made to correct misspelling of a term and, therefore, address an informality. Thus, as the amendments were made to address informalities or are supported by the specification, no new matter has been added and entry thereof is respectfully requested.

Regarding the New Claims

New claims 58 to 83, which depend from claim 4, are supported throughout the specification. In particular, claims 58 to 62 are supported, for example, at page 5, lines 15-19, which discloses that the antibody can be mutated "in a framework region or CDR;" and at page 14, lines 26-28, which discloses exemplary substitutions of "1-3, 3-5 or 5-10 amino acids." Claims 63 to 83 are supported, for example, at page 5, lines 15-19, which discloses that "complemetarity determining region (CDR) from a non-human antibody are grafted into a human framework region" and that "following grafting, one or more amino acids are mutated to human sequences" which can produce a "humanized antibody having increased antigen binding affinity relative to the non-human or grafted antibody;" at page 6, lines 3-7, which discloses exemplary antigen binding affinities greater or less than than either "the donor or recombinant antibody" of "4-fold, 5-fold, 5- to 8-fold, 5- to 10-fold, 8- to 15-fold, 10- to 20-fold, 20- to 40-fold, 20- to 100- fold or greater;" and at page 34, lines 9-13, which discloses non-human antibody "mouse monoclonal antibody 1A6." Thus, as the new claims are supported by the specification, no new matter has been added and entry thereof is respectfully requested.

Regarding the Copies of References filed on PTO-1449

The Examiner indicates that all references have been considered in Applicant's previously filed PTO-1449 except for one reference, denoted PPR. This reference, was not available in the electronic database available to the Examiner and, therefore, was unable to be considered.

Applicants thank the Examiner for considering all references except the reference denoted PPR in the previously filed PTO-1449 forms in spite of the Patent Office's apparent mishandling of the submitted reference copies. Submitted herewith for the examiner's consideration is a copy of the reference denoted PPR. Applicants respectfully request that the Examiner consider this reference in light of the claims under consideration.

I. OBJECTION TO THE DISCLOSURE

The disclosure stands objected to due to the brief description of Figure 2 making reference to colors. Applicant is requested to either submit color photographs or figures, or to amend the specification to describe the black and white drawings.

Submitted herewith are three sets of color photographs of Figures 2A-2C. Submitted herewith are the required petition under 37 C.F.R. §1.84 and fee under 37 C.F.R. §1.17(h) for acceptance of the color photographs. The specification has also been amended as required under 37 C.F.R. §1.84(iv) to include a sentence referencing the color photographs. Accordingly, in view of the submission of color photographs, Applicants respectfully request that the objection to the disclosure be withdrawn.

II. OBJECTION TO VARIOUS CLAIMS

Claims 1, 12, 16, 22 and 47 stand objected to due to various informalities. As set forth above, the claims have been amended in order to address each of the informalities. In particular, claims 1 and 22 have been amended to conform to the elected inventions; the terms "coxsackie" in claim 12 and "intranassaly" in claims 47, 50 and 55 are correctly spelled; and the semicolon after claim 16 has been deleted. Accordingly, in view of the amendments, Applicants respectfully request that the objection to the claims be withdrawn.

III. REJECTIONS UNDER 35 U.S.C. §112

The rejection of claim 12 under 35 U.S.C. §112, first paragraph as allegedly lacking enablement is respectfully traversed. The Examiner acknowledges that the claims are adequately enabled for "rhinovirus infection, coxsackie A virus infection, or respiratory syncytial virus," but, allegedly, "does not reasonably provide enablement for a humanized antibody that inhibits malaria."

Claim 12, as originally filed, is adequately enabled. Solely in order to expedite prosecution of the application, claim 12 has been amended to delete reference to "malaria" As such, the rejection is moot and Applicants respectfully request that the rejection under 35 U.S.C. §112, first paragraph, be withdrawn.

The rejection of claim 3 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is respectfully traversed. The Examiner indicates that claim 3 lacks clear antecedent for claim 2, which recites subsequence of an antibody.

As set forth above, claim 3 has been amended to recite antibody "subsequence" of claim 2. Accordingly, amended claim 3 is clear and definite and Applicants respectfully request that the rejection under 35 U.S.C. §112, second paragraph, be withdrawn.

IV. REJECTIONS UNDER 35 U.S.C. §102 and 103(a)

The rejection of claims 5, 11 to 15 and 34 to 36 under 35 U.S.C. §102(b) as allegedly anticipated by Adair *et al.* (WO 91/16928) is respectfully traversed. The Examiner indicates that Adair *et al.* describe "a humanized chimeric antibody that binds ICAM-1....that comprises all the structural properties instantly recited."

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration (In re Spada, 15 USPQ 2d 1655 (Fed. Cir. 1990), In re Bond, 15 USPQ 2d 1566 (Fed. Cir. 1990).

Claims 5, 11 to 15 and 34 to 36 have been amended as set forth above. The rejection will therefore be addressed in respect to the amended claims.

Claim 5, as amended, and claims 11 to 15 and 34 to 36, which depend from claim 5, recite that the humanized antibody "variable framework region has one or more amino acids of a human consensus variable framework region sequence" and that "the protective efficacy is at least equivalent to mouse monoclonal antibody denoted as 1A6." Adair *et al.* describe antibodies having one or more non-human CDRs grafted into a human immunoglobulin framework that can also include non-human residues (see, for example, page 25, line 21, to page 26, line 11; page 28, lines 1-15; and page 30, lines 17-21), but do not describe a humanized antibody that binds ICAM-1 and inhibits pathogen infection of cells expressing ICAM-1 in which the variable framework region has one or more amino acids of a human consensus variable framework region sequence. Furthermore, the humanized antibodies described by Adair *et al.* bind with less affinity for ICAM-1 than the non-humanized mouse antibody (see, for example, Figures 18-20, and page 49, line 25, to page 50, line 8). Thus, nor do Adair *et al.* describe a humanized antibody having the protective efficacy at least equivalent to mouse monoclonal antibody denoted as 1A6. Consequently, Adair *et al.* fail to teach or suggest each and every element of claims 11 to 15 and 34 to 36.

Accordingly, because Adair *et al.* (WO 91/16928) fail to teach each and every element of claims 5, 11 to 15 and 34 to 36, the reference cannot anticipate these claims. As such, Applicants respectfully request that the rejection under 35 U.S.C. §102(b) over Adair *et al.* (WO 91/16928) be withdrawn.

The rejection of claims 2, 3, 22 and 28 to 33 under 35 U.S.C. §102(b) as allegedly anticipated or under 35 U.S.C. §103(a) as allegedly unpatentable over Colonno *et al.* (EP 459577) is respectfully traversed. The Examiner indicates that Colonno *et al.* allegedly describe "murine monoclonal antibody 1A6, together with the amino acid sequence of its H and L chains, which includes the relevant binding subsequences of HumB, and nucleic acid encoding the subsequences." The Examiner also indicates that if Colonno *et al.* do not anticipate the claimed subsequences that "these subsequences would have been obvious over Colonno *et al.* because Colonno *et al.* provides sequences that comprise the relevant subsequences, and suggests recombinant production and humanizing of the antibody."

Originally filed claims 2, 3, 22 and 28 to 33 are not anticipated, nor would have been obvious, in view of Colonno *et al.* (EP 0459577 A2). Nevertheless, solely in order to expedite

prosecution of the application and without acquiescing to the propriety of the rejection, these claims have been amended as set forth above. The rejection will therefore be addressed in respect to the amended claims.

Amended claims 2, 22 and 28 from which claims 3 and 29 to 33 depend, recite that "the variable framework region of said subsequence has one or more amino acids of a human consensus variable framework region sequence." In contrast, at most Colonno *et al.* mention that humanized antibodies can be produced by "the exchange of mouse constant regions of a mouse antibody molecule, for a human constant region" (see page 4, lines 42-44). However, Colonno *et al.* do not teach or suggest a subsequence of a humanized antibody in which the variable framework region of the subsequence has one or more amino acids of a human consensus variable framework region sequence.

Accordingly, because Colonno *et al.* (EP 0459577 A2) fail to teach or suggest each and every element of claims 2, 3, 22 and 28 to 33, the reference cannot anticipate these claims nor render these claims obvious. As such, Applicants respectfully request that the rejections under 35 U.S.C. §102(b) and §103(a) over Colonno *et al.* (EP 0459577 A2) be withdrawn.

The rejection of claims 5 to 15 and 34 to 39 under 35 U.S.C. §103(a) as allegedly unpatentable over Colonno *et al.* (EP 459577) in view of U.S. Patent No. 5,821,337 to Carter *et al.* (hereinafter referred to as the '337 patent) is respectfully traversed. The Examiner reiterates that Colonno *et al.* allegedly describe "murine monoclonal antibody 1A6....the amino acid sequence of its H and L chains" and allegedly suggest "recombinant production and humanizing the antibody." The Examiner indicates that Colonno *et al.* differs from the claimed invention by "not exemplifying or disclosing the human immunoglobulin sequence to be used to humanize murine antibody 1A6." Allegedly, the '337 patent describes "consensus human immunoglobulin sequences for use in humanizing antibodies from other species for pharmaceutical applications in humans" and, allegedly "the desirability of improving binding properties." Allegedly, it also would have been obvious "to use the human immunoglobulin sequences of Carter et al. in place of the murine sequences in Colonno et al., while retaining the murine CDRR responsible for the ICAM-1 specificity of antibody 1A6 of Colonno et al. and to improve binding properties" allegedly "because Colonno et al. specifically suggest humanization of the antibody for use in....applications such as preventing HRV infection."

Originally filed claims 5 to 15 and 34 to 39 would not have been obvious, in view of Colonno *et al.* (EP 0459577 A2) or U.S. Patent No. 5,821,337 to Carter *et al.* alone, or in combination. Nevertheless, solely in order to expedite prosecution of the application and without acquiescing to the propriety of the rejection, these claims have been amended as set forth above. The rejection will therefore be addressed in respect to the amended claims.

In order for a rejection to be proper under 35 U.S.C. §103, *inter alia*, there must have been a motivation to modify or combine the references at the time of the invention; the combination of references must teach or suggest each and every element of the claimed invention; and there must have been a reasonable expectation of success at the time of the invention. Both the teaching or suggestion to make the claimed combination and the reasonable expectation of success must be found in the prior art, not in Applicants' disclosure. See, e.g., *In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991) and *In re O'Farrell*, 853 F.2d 894, 903-904 (Fed. Cir. 1988). Here, *inter alia*, there would not have been a motivation to modify the cited references to obtain the claimed compositions, nor a reasonable expectation of success of producing the claimed compositions, in view of Colonno *et al.* (EP 0459577 A2) or U.S. Patent No. 5,821,337 alone or in combination at the time of the invention.

Amended claims 5 to 15 and 34 to 39, recite, *inter alia*, that the humanized antibody that binds ICAM-1 has a variable framework region with one or more amino acids of a human consensus variable framework region sequence. As an example, humanized antibody HumB Vh framework positions 71 and 94 are amino acids not found in mouse 1A6 antibody nor in the human framework acceptor. Instead, both amino acids at positions 71 and 94 (serine and alanine, respectively) are based upon a human consensus variable framework region sequence.

As discussed, Colonno *et al.* do not teach or suggest a humanized antibody in which the variable framework region has one or more amino acids of a human consensus variable framework region sequence. In this regard, Colonno *et al.* do not teach or suggest any human framework region sequence, let alone a sequence having one or more amino acids of a human consensus variable framework region sequence.

The '337 patent describes humanizing antibodies using a CDR sequence of an import, non-human antibody and the FR sequence of a human antibody (column 6, lines 24-25). However, the '337 patent indicates that after the CDRs are grafted into the human framework sequence, variable sequences in the grafted antibody can be substituted. For example, the '337

patent indicates that residues from non-human antibody from which the CDRs were obtained can be used for substitutions in the framework region (column 6, lines 15 to 17 and 24-25; column 23, lines 51-64; column 62, lines 17-30). Humanized antibodies can have replacements of non-human import residues at positions within the CDR residues (column 21, lines 53-67). However, the '337 patent does not teach or suggest that after grafting of CDRs into human framework acceptor that the human framework sequence region be substituted with one or more amino acids based upon a human consensus variable framework region sequence. Absent such a teaching or suggestion, the claimed compositions would not have been produced.

Amended claims 5 to 15 and 34 to 39, also recite, *inter alia*, that the humanized antibody that binds ICAM-1 have a protective efficacy at least equivalent to mouse monoclonal antibody denoted as 1A6. However, no humanized antibody produced in the '337 patent achieves an antigen binding affinity without either replacing murine CDR residues with human residues, replacing human framework residues with non-human (murine) framework residues, or both. In this regard, Applicants respectfully direct the Examiner's attention to Table 3 (column 55), which describes a total of eight humanized antibodies against her2. However, seven of these antibodies, huMab4D5-2 through huMab4D5-8, have mutations in which murine CDR residues were replaced with human residues or human framework residues were replaced with murine residues (see, for example column 53, lines 2-15 and Table 3, huMab4D5-1 through 5-8). The antibody having the most human to non-human framework region substitutions has the greatest affinity (K_D) (compare huMab4D5-8 to muMab4D5). The data in the '337 patent therefore indicates that to generate a humanized antibody with comparable antigen binding affinity as non-humanized donor antibody to either replace non-human CDR residues with human residues, replace human framework residues with non-human framework residues, or do both.

Given that the '337 patent teaches the skilled artisan to replace non-human CDR residues with human residues human framework region residues with non-human residues from the donor antibody in order to obtain a humanized antibody with comparable antigen affinity, the skilled artisan would not have produced a humanized antibody having equivalent protective efficacy as non-humanized antibody by substituting one or more amino acids of a human consensus framework region sequence into the humanized antibody. As such, the skilled artisan would not have been motivated to substitute the humanized antibody with one or more amino acids of a human consensus framework region sequence, as in claims 5 to 15 and 34 to 39. Consequently,

in view of the '337 patent the skilled artisan would not have been motivated to produce claims 5 to 15 and 34 to 39.

Furthermore, the '337 patent teaches away from producing the claimed compositions. As discussed above, the '337 patent repeatedly describes substituting non-human CDR residues with human residues or human framework region residues with non-human residues. The '337 patent humanized antibodies all either have human substitutions in the CDRs, non-human substitutions in the framework regions, or both. Thus, given that the '337 patent repeatedly describes substituting human residues in the CDRs or non-human residues in the framework regions of the humanized antibodies or both, the '337 patent teaches away from producing the compositions of claims 5 to 15 and 34 to 39.

As additional evidence that the '337 patent teaches away from producing the claimed compositions, Applicants submit herewith Exhibit A (Carter *et al.*, Proc. Natl. Acad. Sci. USA 89:4285 (1992)). Exhibit A is a publication describing the same anti-her2 antibodies as the '337 patent. Table 1 in Exhibit A (page 4287) is analogous to Table 3 in the '337 patent. The authors in Exhibit A state that the most potent anti-her2 humanized antibody had five FR residues mutated to murine residues (page 4288, first column, first full paragraph). The authors further state that the least potent anti-her2 antibody, humAb44D5-1 was the most humanized (page 4288, first column, first full paragraph). Thus, given that Exhibit A indicates that the most humanized antibody was the least potent antibody, Exhibit A teaches the skilled artisan away from producing a humanized antibody having a variable framework region with one or more amino acids of a human consensus variable framework region sequence and having a binding affinity comparable to the donor non-human antibody. Consequently, in view of Exhibit A the skilled artisan would not have been motivated to produce the claimed humanized antibodies.

As further evidence that the claimed compositions would not have been obvious, Applicants submit herewith Exhibit B (Presta *et al.*, J. Immunol. 151:2623 (1993)). Exhibit B is a publication describing anti-IgE antibodies. Again, the anti-IgE antibodies described have human framework residues replaced with non-human residues (see, page 2626, second column, last paragraph and page 2625, Figure 1). In order to obtain a humanized anti-IgE antibody having comparable binding affinity as the donor non-human anti-IgE antibody MaE11, five framework residues were replaced with murine residues (see, page 2631, first column, second paragraph). Thus, given that Exhibit B indicates that anti-IgE humanized antibody with comparable affinity

to donor non-human anti-IgE antibody required five framework residues to be replaced with murine residues, Exhibit B also teaches the skilled artisan away from producing a humanized antibody having a variable framework region with one or more amino acids of a human consensus variable framework region sequence and having a binding affinity comparable to the donor non-human antibody. Consequently, in view of Exhibit B the skilled artisan would not have been motivated to produce the claimed humanized antibodies.

Moreover, in view of the '337 patent, one skilled in the art at the time of the invention would not have had a reasonable expectation of success of producing a humanized antibody having a protective efficacy at least equivalent to the non-human donor antibody as in claims 5 to 15 and 34 to 39. Again, the '337 patent teaches that in order to obtain a humanized antibody with comparable affinity as the donor non-human antibody the murine CDRs must be replaced with human residues, human framework residues must be replaced with residues from the corresponding non-human donor, or both. All humanized antibodies produced in the '337 patent having antigen binding affinity even close to the donor antibody had non-human CDR residues mutated to human residues, human framework residues mutated to non-human residues, or both (column 21, lines 53-55; column 62, lines 13-30; and Table 3). Thus, given the description of the '337 patent the skilled artisan would not have reasonably expected that a humanized antibody with comparable affinity as donor antibody could be obtained by producing a humanized antibody having a variable framework region with one or more amino acids of a human consensus variable framework region sequence, as in claims 5 to 15 and 34 to 39. As such, the '337 patent does not provide a reasonable expectation of success of producing claims 5 to 15 and 34 to 39.

Thus, given that neither Collono *et al.* nor the '337 patent teaches or suggests a humanized antibody in which the human framework sequence region has one or more amino acids of a human consensus variable framework region sequence, and that the '337 patent teaches away from producing the claimed compositions one skilled in the art would not have been motivated to produce such a humanized antibody. Furthermore, given the fact that the '337 patent describes mutating the humanized antibodies by replacing non-human CDRs with human residues, replacing human framework residues with non-human donor residues, or both in order to obtain antibodies with comparable affinity as non-humanized donor, there would not have been a reasonable expectation of success in producing the humanized antibodies of claim 5.

Absent the requisite motivation and reasonable expectation of success, the rejection of claims 5 to 15 and 34 to 39 under 35 U.S.C. §103(a) is improper and must be withdrawn.

The rejection of claims 16 to 21 and 40 to 57 under 35 U.S.C. §103(a) as allegedly unpatentable over Colonno *et al.* (EP 459577) and U.S. Patent No. 5,821,337 (the '337 patent) in view of Terskikh *et al.* (Proc. Natl. Acad. Sci. USA 94:1663 (1997)) is respectfully traversed. The Examiner acknowledges that neither Colonno *et al.* nor the '337 patent teach multimerization of the humanized antibody. However, allegedly Terskikh *et al.* describe "the advantages of multivalency achieved by combining of specific binding molecules with the same or different binding specificities, in particular, to gain the advantage of high avidity."

Originally filed claims 16 to 21 and 40 to 57 would not have been obvious, in view of Colonno *et al.* (EP 0459577 A2), U.S. Patent No. 5,821,337 or Terskikh *et al.* alone, or in combination. Nevertheless, solely in order to expedite prosecution of the application and without acquiescing to the propriety of the rejection, these claims have been amended as set forth above. The rejection will therefore be addressed in respect to the amended claims.

Colonno *et al.* and the '337 patent have been discussed above. In brief, neither Colonno *et al.* nor the '337 patent provide the requisite motivation or expectation of success of producing the antibodies of claim 5, as is required for a rejection under 35 U.S.C. §103(a) to be proper. Furthermore, the '337 patent teaches away from producing the antibodies of claim 5, from which claims 16 to 21 and 40 to 57 ultimately depend.

Terskikh *et al.* do not provide that which is missing from Colonno *et al.* and the '337 patent. For example, *inter alia*, Terskikh *et al.* do not teach or suggest, a humanized antibody in which the human framework sequence region has one or more amino acids of a human consensus variable framework region sequence. Thus, Terskikh *et al.* do not provide the skilled artisan with the requisite motivation to produce the humanized antibodies of claim 5. Furthermore, Terskikh *et al.* is silent on producing humanized antibodies and, therefore, do not provide a reasonable expectation of success of producing the humanized antibodies of claim 5. Absent the requisite motivation and reasonable expectation of success, the rejection of claims 16 to 21 and 40 to 57 under 35 U.S.C. §103(a) is improper and must be withdrawn.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 1 to 83 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 509-4065.

Please charge any additional fees, or make any credits, to Deposit Account No. 03-3975.

Respectfully submitted,

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ages of 1 to 5, 5 to 10 or 10 to 18. In still another aspect, the antibody, subsequence, multimer, variant or modified form binds to an antigen present on the surface of the cell (e.g., ICAM-1). In various additional aspects, the humanized antibody is administered locally, via inhalation or intranasaly.

The invention also provides methods of inhibiting HRV infection, inhibiting HRV progression or treating HRV infection of a subject. In one embodiment, a method includes administering to a subject having or at risk of having HRV infection an amount of a humanized antibody, subsequence, multimer, variant or modified form effective to inhibit HRV infection, inhibit HRV progression or treat HRV infection of the subject. In one aspect, the subject has or is at risk of having asthma. In another aspect, the subject is a newborn or between the ages of 1 to 5, 5 to 10 or 10 to 18. In various additional aspects, the humanized antibody is administered locally, via inhalation or intranasaly.

The invention additionally provides methods of decreasing or inhibiting one or more symptoms of the common cold in a subject. In one embodiment, a method includes administering to a subject having a common cold an amount of a humanized antibody, subsequence, multimer, variant or modified form effective to decrease or inhibit one or more symptoms of the common cold in the subject. In one aspect, the subject has or is at risk of having asthma. In another aspect, the subject is a newborn or between the ages of 1 to 5, 5 to 10 or 10 to 18. In various additional aspects, the humanized antibody is administered locally, via inhalation or intranasaly.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence of murine 1A6 antibody and human consensus sequence of heavy chain subgroup III (Humiii) and light chain kappa subgroup I. (Asterisks denote amino acid differences between human and mouse sequence. CDR amino acids are in bold face. (SEQ ID NOS:37 AND 39, RESPECTIVELY).

Figure 2 shows a molecular model of humanized 1A6 (Hum B). **(A)** Side view of humB variable domains. Yellow: V_H; green: V_L; pale blue: CDRs; red: the six high risk "Vernier zone" residues and the V_H 60-64. **(B)** Top view of humB variable domains. Yellow: V_H; green: V_L; pale blue: CDRs; red: the six high risk "Vernier zone" residues

and the V_H 60-64. **(C)** V_L49 and its surrounding residues. Magenta: ICAM-1; red: V_H and V_L residues; blue: residues at V_H-V_L interphase; purple: W102 in V_H.

Figure 3 shows the amino acid sequence of murine 1A6 antibody, humanized 1A6 (HumB) and human consensus sequences of heavy chain subgroup III (Humiii) and light chain kappa subgroup I. Asterisks and bold face amino acids are as previously indicated. (SEQ ID NOS:37 AND 39, RESPECTIVELY) (SEQ ID NOS: 38 AND 40, RESPECTIVELY)

Figure 4 shows the cDNA sequence of humanized scFV3 (HumA) antibody. Restriction sites are indicated by underlining. (SEQ ID NO: 47)

Figure 5 shows protection from HRV15 infection with mouse 1A6 scFv antibody and humanized 1A6 scFv antibody HumA, HumB, HumC, HumD, HumF, HumH and HumI.

DETAILED DESCRIPTION

The present invention is based, at least in part, upon producing humanized antibodies. More particularly, complementarity determining region (CDR) from a non-human antibody are grafted into a human framework region. Following grafting, one or more amino acids of the antibody are mutated to human sequences. For example, mutating a murine amino acid to a human amino acid in a framework region or CDR of the grafted antibody can produce a humanized antibody having increased antigen binding affinity relative to the non-human or grafted antibody. Humanized antibodies are not immunogenic or are less immunogenic than non-human antibodies when administered to human subjects. Therefore, humanized antibodies are useful in a variety of therapeutic and diagnostic applications. For example, as exemplified herein, a humanized antibody of the invention protects cells from HRV infection, a virus that can cause the common cold, and other associated disorders (e.g. otitis media, bronchitis, sinusitis etc.).

Thus, in accordance with the invention, there are provided humanized antibodies. In one embodiment, a humanized antibody binds to ICAM-1. In one aspect, a humanized antibody that binds ICAM-1 protects against pathogen infection of cells expressing ICAM-1. In other aspects, a humanized antibody is selected from any of SEQ ID NO:1 and 3 (HumA); SEQ ID NO:5 and 7 (HumB); SEQ ID NO:9 and 11 (HumC); SEQ ID NO:13 and 15 (HumD); SEQ ID NO:17 and 19 (HumE); SEQ ID NO:21 and 23 (HumF);

K30 is not involved directly in antigen binding, this stabilizing change is predicted to be either neutral or beneficial.

VH 71 and VH94:

Structural inspection indicated that both of these positions require a residue with a small side chain for maintenance of proper antibody conformation. Therefore, the human consensus residue at this position, arginine, is not appropriate. Serine and glycine were selected for position 71.

According to Chothia et al., residues at VH94 is related with the canonical structure of H1 or CDR1 (defined as VH26-VH32). The CDR1 of 1A6 belongs to the canonical structure 1 and family 1 (Chothia and Lesk (1987) J. Mol. Biol. 186:651-663; Chothia et al. (1992) J. Mol. Biol. 227:799-817; Chothia et al. (1989) Nature 342:877-883). Corresponding to this canonical structure, human sequences showed three possible residues at VH94 position: arginine, threonine or alanine (Chothia et al. (1992) J. Mol. Biol. 227:799-817). Since arginine is not appropriate for this particular antibody, alanine, threonine and another small residue, aspartic acid were chosen.

Finally, molecular model building indicates that a portion of the CDR2 in the VH domain, VH60-64, does not have direct contact with the antigen. Therefore mouse residues at these positions (DPKVQ) can be replaced by human residues ADSVK. (PIECE of SEQ ID NO: 17) (PIECE of SEQ ID NO: 1)

Example 2

This example describes the preparation of several humanized scFv expression constructs.

The humanized scFv A (HumA) cDNA (FIG. 4) (SEQ ID NO: 47) containing 750 bb was synthesized using a series of overlapping oligonucleotides. These overlapping oligonucleotides (Table 1) were designed to encode the amino acids of the variable region of the heavy (V_H) and light (V_L) chains linked by a linker($(G_4S)_4$) with a Bam H1 site. The heavy chain and light chain were cloned separately in TOPO 2.1 vector. After (SEQ ID NO: 96) DNA sequencing conformation, the heavy and light chain were subcloned into expression vector (pBAD/pIII A) to form full length DNA.

Table 1. Oligonucleotides for humanized scFvs

Oligonucleotides for the light (V_H) chain of HumA:

AVL-1: (SEQ ID NO: 48)
CGAACCATGGGCGATATCCAGATGACCCAATCTCCGTCTAGCCTGAGCGCCAGTGTTGGTG

AVL-2: (SEQ ID NO: 49)
GTGAAGATTATTACTGATAGATTGGCTGGCGCGGCAAGTAATGGTAACTCGATCACCAACAC
TGGCGCTCAG

AVL-3: (SEQ ID NO: 50)
CTATCAGTAATAATCTTCACTGGTATCAACAAAAACCGGGTAAAGCTCCGAAACTTCTTATCT
ATCACGCC

AVL-4: (SEQ ID NO: 51)
CCCGAGCCAGAGCCAGAGAAGCGGCTCGGAACGCCGCTAATGCTCTGAGAGGCGTGATAG
ATAAGAAG

AVL-5: (SEQ ID NO: 52)
CTCTGGCTCTGGCTCGGGCACGGACTTTACCCTTACCATCAGCTCTCTTCAGCCGGAAGAC
TTTGCCACC

AVL-6: (SEQ ID NO: 53)
CCTTGACCGAAGGTATACGGCCAGCTATTAGACTGCTGACAATAATAGGTGGCAAAGTCTTC
CGGC

AVL-7: (SEQ ID NO: 54)
GTATACCTTCGGTCAAGGTACCAAGGTCGAGATTAAGCGCGGCGGTGGCGGTTCTGGTGGC
GGTGGTAGCG

AVL-8: CGAACCATGGGCGATATCCAGATGACCCAATC
(SEQ ID NO: 55)

AVL-9: CGGATCCACCGCCACCGCTACCACCGCCACCAG
(SEQ ID NO: 56)

Oligonucleotides for the heavy (V_H) chain of HumA:

AVH-1: (SEQ ID NO: 57)
GGTGGCGGTGGATCCGGTGGCGGTGGCAGCGAAGTTCAACTTGTTGAGTCTGGTGGCGGT
CTGGTTCAGCCGG

AVH-2: (SEQ ID NO: 58)
GTCCTTAATGTTGAAACCGCTTGCTGCGCAAGACAGGCGCAGAGAGCCACCCGGCTGAACC
AGACCGCCAC

AVH-3: (SEQ ID NO: 59)
GGTTTCAACATTAAGGACACCTACATCCATTGGGTGAGGCAAGCTCCGGGTAAGGGTCTGG
AGTGGG

AVH-4: (SEQ ID NO: 60)
GGCCCTTCACGCTGTCAGCGTAAATGGTGTTGTCGTTTGCCGGGTCGATACGTGCCACCCA
CTCCAGACCCTTACC

AVH-5: (SEQ ID NO: 61)
CGCTGACAGCGTGAAGGGCCGTTTTACTATTTCTAGCGACGACTCTAAGAACACCGCGTAC
CTTCAGATGAACTCTCTGCG

AVH-6: (SEQ ID NO: 62)
CCAGTAGCCAGAGTCCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG

AVH-7: (SEQ ID NO: 63)
GGACTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC

AVH-8: GGTGGCGGTGGATCCGGT
(SEQ ID NO: 64)

AVH-9: GGGTTAACCAGAAGAGACGG
(SEQ ID NO: 65)

Oligonucleotides for making other human scFv (Hum B-I):

BVH-6: (SEQ ID NO: 66)
CCAGTAGCCAGAGGCCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG

BVH-7: (SEQ ID NO: 67)
GGCCTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC

CVH-5: (SEQ ID NO: 68)
CGCTGACAGCGTGAAGGGCCGTTTTACTATTTCTGGCGACGACTCTAAGAACACCGCGTAC
CTTCAGATGAACTCTCTGCG

CVH-6: (SEQ ID NO: 69)
CCAGTAGCCAGAGGTCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG

CVH-7: (SEQ ID NO: 70)
GACCTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC

DVH-6: (SEQ ID NO: 71)
CCAGTAGCCAGAGGTCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG

DVH-7: (SEQ ID NO: 72)
GACCTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC

EVH-4: (SEQ ID NO: 73)
GGCCCTGCACCTTCGGATCGTAAATGGTGTTGTCGTTTGCCGGGTCGATACGTGCCACCCA
CTCCAGACCCTTACC

EVH-5: (SEQ ID NO: 74)
CGATCCGAAGGTGCAGGGCCGTTTTACTATTTCTGCGGACGACTCTAAGAACACCGCGTAC
CTTCAGATGAACTCTCTGCG

EVH-6: (SEQ ID NO: 75)
CCAGTAGCCAGAGGTCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG

EVH-7: (SEQ ID NO: 76)
GACCTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC

FVH-6: (SEQ ID NO: 77)
CCAGTAGCCAGAGGTCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG

FVH-7: (SEQ ID NO: 78)
GACCTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC

GVL-3: (SEQ ID NO: 79)
CTATCAGTAATAATCTTCACTGGTATCAACAAAAACCGGGTAAAGCTCCGAAACTTCTTATCA
AACACGCC

GVL-4: (SEQ ID NO: 80)
CCCGAGCCAGAGCCAGAGAAGCGGCTCGGAACGCCGCTAATGCTCTGAGAGGCGTGAAAG
ATAAGAAG

GVH-5: (SEQ ID NO: 81)
CGCTGACAGCGTGAAGGGCCGTTTTACTATTTCTGCGGACGACTCTAAGAACACCGCGTAC
CTTCAGATGAACTCTCTGCG

GVH-6: (SEQ ID NO: 82)
CCAGTAGCCAGAGGTCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG

GVH-7: (SEQ ID NO: 83)
GACCTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC
HVL-3: (SEQ ID NO: 84)
CTATCAGTAATAATCTTCACTGGTATCAACAAAAACCGGGTAAAGCTCCGAAACTTCTTATCA
AACACGCC
HVL-4: (SEQ ID NO: 85)
CCCGAGCCAGAGCCAGAGAAGCGGCTCGGAACGCCGCTAATGCTCTGAGAGGCGTGAAAG
ATAAGAAG
HVH-4: (SEQ ID NO: 86)
GGCCCTGCACCTTCGGATCGTAAATGGTGTTGTCGTTTGCCGGGTCGATACGTGCCACCCA
CTCCAGACCCTTACC
HVH-5: (SEQ ID NO: 87)
CGATCCGAAGGTGCAGGGCCGTTTTACTATTTCTGCGGACGACTCTAAGAACACCGCGTAC
CTTCAGATGAACTCTCTGCG
HVH-6: (SEQ ID NO: 88)
CCAGTAGCCAGAGGTCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG
HVH-7: (SEQ ID NO: 89)
GACCTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC
IVL-3: (SEQ ID NO: 90)
CTATCAGTAATAATCTTCACTGGTATCAACAAAAACCGGGTAAAGCTCCGAAACTTCTTATCA
AACACGCC
IVL-4: (SEQ ID NO: 91)
CCCGAGCCAGAGCCAGAGAAGCGGCTCGGAACGCCGCTAATGCTCTGAGAGGCGTGAAAG
ATAAGAAG
IVH-4: (SEQ ID NO: 92)
GGCCCTGCACCTTCGGATCGTAAATGGTGTTGTCGTTTGCCGGGTCGATACGTGCCACCCA
CTCCAGACCCTTACC
IVH-5: (SEQ ID NO: 93)
CGATCCGAAGGTGCAGGGCCGTTTTACTATGTCTGCGGACACCTCTAAGAACACCGCGTAC
CTTCAGATGAACTCTCTGCG
IVH-6: (SEQ ID NO: 94)
CCAGTAGCCAGAGGTCGTGCAGTAGTAGACGGCGGTGTCCTCGGCACGCAGAGAGTTCAT
CTGAAGG
IVH-7: (SEQ ID NO: 95)
GACCTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGTCTCTTCTGGT
TAAC

Molecular model building enabled synthesis of 9 versions of humanized antibodies in the form of scFv (HumA-HumI, summarized in Tables 2 and 3). Four of the humanized antibodies, HumA-HumD, do not have parental mouse framework residues, and five of them, HumE-HumI, contain various number of parental mouse residues in the framework. The sequence of HumB is compared against parental mouse 1A6 and human consensus framework in FIG. 3.

Table 2. Humanization Constructs

Position	L49	H37	H60-64	H69	H71	H73	H94
Human / Mouse	Y/K	V/M	ADSVK/DPKVQ	I/M	R/A	D/T	R/T
HumA	Y	V	ADSVK	I	S	D	D
HumB	Y	V	ADSVK	I	S	D	A
HumC	Y	V	ADSVK	I	G	D	T
HumD	Y	V	ADSVK	I	S	D	T
HumE	Y	V	DPKVQ	I	A	D	T
HumF	Y	V	ADSVK	I	A	D	T
HumG	K	V	ADSVK	I	A	D	T
HumH	K	V	DPKVQ	I	A	D	T
HumI	K	M	DPKVQ	M	A	T	T

ADSVK IS PIECE OF (SEQ ID NO:1), DPKVQ IS A PIECE OF (SEQ ID NO:17).

Table 3. Amino Acid Sequences of Humanized Antibody

Hum A:

vH Domain (SEQ ID NO:1)

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Ala Asp Ser Val Lys Gly) Arg Phe Thr Ile Ser Ser Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Asp (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 3

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

Hum B:
VH Domain (SEQ ID NO: 5)
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Ala Asp Ser Val Lys Gly) Arg Phe Thr Ile Ser Ser Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Ala (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 7)
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

Hum C:
VH Domain (SEQ ID NO: 9)
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Ala Asp Ser Val Lys Gly) Arg Phe Thr Ile Ser Gly Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 11)
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr

Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

Hum D:
VH Domain (SEQ ID NO: 13)
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Ala Asp Ser Val Lys Gly) Arg Phe Thr Ile Ser Ser Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 15)
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

Hum E:
VH Domain (SEQ ID NO: 17)
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Asp Pro Lys Val Gln Gly) Arg Phe Thr Ile Ser Ala Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 19)
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

Hum F:
VH Domain (SEQ ID NO: 21)
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Ala Asp Ser Val Lys Gly) Arg Phe Thr Ile Ser Ala Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 23)
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

Hum G:
VH Domain (SEQ ID NO: 25)
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Ala Asp Ser Val Lys Gly) Arg Phe Thr Ile Ser Ala Asp Asp Ser Lys Asn Thr Ala Tyr Leu

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 27)

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Lys (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

Hum H:

VH Domain (SEQ ID NO: 29)

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Asp Pro Lys Val Gln Gly) Arg Phe Thr Ile Ser Ala Asp Asp Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 31)

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Lys (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

Hum I:

VH Domain (SEQ ID NO: 33)

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser (Gly Phe Asn Ile Lys Asp Thr Tyr Ile His) Trp Met Arg Gln Ala

Pro Gly Lys Gly Leu Glu Trp Val Ala (Arg Ile Asp Pro Ala Asn Asp Asn Thr Ile Tyr Asp Pro Lys Val Gln Gly) Arg Phe Thr Met Ser Ala Asp Thr Ser Lys Asn Thr Ala Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr (Ser Gly Tyr Trp Phe Ala Tyr) Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

VL Domain (SEQ ID NO: 35)
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys (Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His) Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Lys (His Ala Ser Gln Ser Ile Ser) Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys (Gln Gln Ser Asn Ser Trp Pro Tyr Thr) Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

The CDR residues are included within brackets.

Example 3

This example describes expression and purification of humanized 1A6 single chain antibody proteins.

For production of the humanized 1A6 scFv, TOP10 cells transformed with desired expression construct were grown in shaker flasks in TB medium (Bio 101) until they reached an OD_{600} of 0.8. Protein expression was induced with 0.02% arabinose for eighteen hours at room temperature. Cells were pelleted by centrifugation at 4,000 g for 15 minutes. Cell pellets were resuspended in $1/50^{th}$ volume of lysis buffer (20 mM sodium phosphate, 1% Triton X-100, 500 mM NaCl, 40 mM imidazole, 2 mM 2-mercaptoethanol), 0.2 mM PMSF, 1mg/ml lysozyme and incubated on ice for 30 minutes. The cell suspension was sonicated and another aliquot of PMSF was added. The cell debris was pelleted by centrifugation at 12,000 x g and the clarified sonicate was filtered and fractionated by metal affinity chromatography. Induced histidine-tagged proteins were bound to a Hi Trap™ metal chelating column (Amersham/Pharmacia) equilibrated with Ni^{2+} according to the manufacturer's instructions. The column was then washed with four column volumes of buffer consisting of 100 mM imidazole, 20 mM sodium phosphate, pH 7.4, 500 mM NaCl. Fractions of proteins eluted from the column in 500